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Acetyl-CoA carboxylase-α inhibitor TOFA induces human cancer cell apoptosis ☆

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ABSTRACT

Acetyl-CoA carboxylase-α (ACCA) is a rate-limiting enzyme in long chain fatty acid synthesis, playing a critical role in cellular energy storage and lipid synthesis. *ACCA* is upregulated in multiple types of human cancers and small interfering RNA-mediated *ACCA* silencing in human breast and prostate cancer cells results in oxidative stress and apoptosis. This study reports for the first time that TOFA (5-tetradecyloxy-2-furoic acid), an allosteric inhibitor of ACCA, is cytotoxic to lung cancer cells NCI-H460 and colon carcinoma cells HCT-8 and HCT-15, with an IC_{50} at approximately 5.0, 5.0, and 4.5 μg/ml, respectively. TOFA at 1.0–20.0 μg/ml effectively blocked fatty acid synthesis and induced cell death in a dose-dependent manner. The cell death was characterized with PARP cleavage, DNA fragmentation, and annexin-V staining, all of which are the features of the apoptosis. Supplementing simultaneously the cells with palmitic acids (100 μM), the end-products of the fatty acid synthesis pathway, prevented the apoptosis induced by TOFA. Taken together, these data suggest that TOFA is a potent cytotoxic agent to lung and colon cancer cells, inducing apoptosis through disturbing their fatty acid synthesis.

Introduction

Acetyl-CoA carboxylase (ACC) is a biotin-dependent, multi-domain enzyme with biotin carboxylase (BC) and carboxyltransferase (CT) activity in most eukaryotes. ACC catalyzes the irreversible carboxylation of acetyl-CoA via a two-step mechanism [1–3]. In sequence, BC catalyzes an ATP-dependent carboxylation of biotin with bicarbonate as a CO_2 donor, and CT promotes the carboxyl transfer from biotin to acetyl-CoA to form malonyl-CoA. The malonyl-CoA is a dual functional compound that serves as a substrate of fatty acid synthase (FAS) for acyl chain elongation and as an inhibitor of carnitine palmitoyltransferase 1 (CPT-1), a key enzyme in long chain fatty acid β-oxidation. This feature of the malonyl-CoA ensures that the two processes, fatty acid synthesis and oxidation, do not occur simultaneously [3,4].

Two ACC isoforms, ACC-α (ACCA) and ACC-β (ACCB), have been identified in mammalians, which are encoded by different genes [2,5]. In normal tissues, ACCA is enriched in lipogenic tissues, such as the liver and adipose, as well as the mammary gland during lactation, but ACCB is expressed abundantly in the heart, skeletal muscle and liver, the tissues with active fatty acid oxidation [2,6]. ACC activity is strictly controlled at the transcriptional and post-translational levels, as well as by a metabolite-mediated allosteric mechanism [3,7,8]. Reversible phosphorylation, characterized with deactivation by phosphorylation, is the major post-translational regulatory mechanism; and insulin, glucagon, and many growth factors are involved in the regulation of the phosphorylation–dephosphorylation switch of the ACCA. In addition, breast cancer 1 (BRCA1), a tumor suppressor protein, blocks Ser^{79} residue from dephosphorylation through direct association of its BRCT tandem domain at C-terminus with the ACCA; and aldo–keto reductase family 1 member B10 (AKR1B10) binds to the ACCA, blocking its degradation through the ubiquitination-proteasome pathway [9,10].

Interestingly, ACCA is upregulated in many types of human cancers, such as breast and liver carcinomas, and likely contributes to the growth and proliferation of cancer cells by stimulating lipogenesis [1,9,11,12]. In prostate and breast cancer cells, RNAi-mediated silencing of *ACCA* inhibits fatty acid synthesis, arrests cell cycle, and induces caspase-mediated apoptosis [13,14]. Therefore, the elevated expression and critical role of the ACCA in cell growth and survival lead to a notion that this protein may be a valuable target for anticancer therapy. TOFA (5-tetradecyloxy-2-furoic acid)

Abbreviations: ACCA, acetyl-CoA carboxylase-α; ACCB, acetyl-CoA carboxylase-β; AKR1B10, aldo–keto reductase family 1 B10; BC, biotin carboxylase; CPT-1, carnitine palmitoyltransferase 1; CT, carboxyltransferase; FAS, fatty acid synthetase; PARP, Poly (ADP-ribose) polymerase; TOFA, 5-tetradecyloxy-2-furoic acid.

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is an allosteric inhibitor of the ACCA. Inside the cell, such as adipocytes and hepatocytes, TOFA is converted to TOFyl-CoA (5-tetradecyloxy-2-furoyl-CoA), exerting an allosteric inhibition on ACCA [15,16]. In this study, we evaluated the effects of TOFA on fatty acid synthesis and survival of human lung cancer cells NCI-H460 and colonic carcinoma cells HCT-8 and HCT-15, and found for the first time that TOFA induces the apoptosis of these cancer cells in a dose-dependent manner.

Materials and methods

Cell culture. NCI-H460, human lung cancer cells, and HCT-8 and HCT-15, human colonic carcinoma cells, were purchased from American Type Culture Collection (Manassas, VA) and maintained in RPMI-1640 medium (Hyclone, UT) containing 10% fetal bovine serum and 2 mM glutamine at 37 °C, 5% CO_2. For TOFA (Sigma, MO) exposures, cells were grown at 60% of confluence, and TOFA was applied at the indicated concentrations for 24 h.

ACCA silencing. Scrambled and *ACCA* specific siRNAs [14] were chemically synthesized (Ambion, TX) and delivered into NCI-H460, HCT-8 and HCT-15 cells ($3.5 \times 10^{4-5}$ in Opti-MEM I medium) as described previously [17].

Palmitic acid rescues. Palmitic acids were provided with a bovine serum albumin (BSA) complex [14]. Briefly, 4 volumes of 4% fatty acid-free BSA (Hyclone, UT) in 0.9% NaCl were mixed with 1 volume of 5 mM palmitate (Sigma, MO) in ethanol and incubated at 37 °C for 1 h to form 1 mM palmitate–BSA complex. Rescues were exerted by adding the palmitate–BSA complex (100 μM) to the TOFA-treated cells at the indicated time.

MTT assay. TOFA cytotoxicity was assessed using a MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole] Cell Proliferation kit (Roche, IN) as described previously [18]. Briefly, cells (5000/well) were seeded in 96-well plates overnight and then exposed to TOFA at indicated concentrations for 72 h. Viable cells were detected.

Western blot. Cells were lysed in complete lysis buffer (Roche, IN), followed by centrifugation at 14,000 rpm for 15 min to collect the soluble proteins. Proteins (100 μg) were separated on 8% SDS–PAGE and then subjected for immuno-blotting analysis as previous described [9].

Apoptosis. Apoptosis was evaluated by PARP cleavage, DNA fragmentation, and annexin-V staining and FACScan analysis. PARP cleavage was detected by Western blot as described above; DNA fragmentation was examined as addressed previously [19]; and annexin-V staining and FACScan was performed as previously described [17].

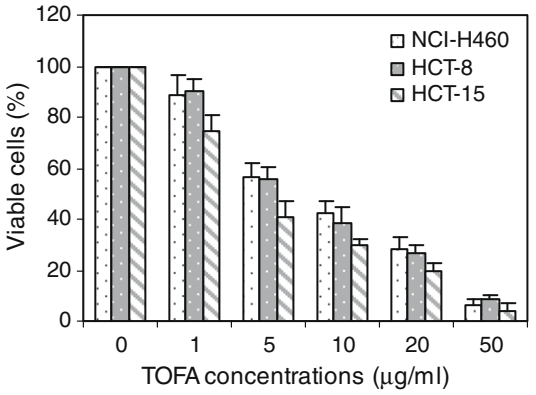

Fig. 1. Cytotoxicity of TOFA. NCI-H460, HCT-8 and HCT-15 cells were seeded into 96-well plates at 5000 cells/well. After incubation overnight, cells were fed with fresh medium containing TOFA at indicated concentrations, followed by continuing incubation for 72 h. Thereafter, viable cells were assessed by MTT cell proliferation kit following the manufacture's instructions. Data indicates the mean ± SD from three independent measurements.

Fatty acid synthesis. Cells were pulsed with 1 μCi 2-^{14}C-labeled acetate (53 mCi/mmol; Amersham Biosciences, NJ) per well of 12-well plates for 4 h at 37 °C, 5% CO_2, followed by lipid synthesis analysis as previously described [9].

Statistical analysis. Student's *t*-test was used for statistically significant analysis with INSTAT statistical analysis package (Graph Pad Software, CA). Significance was defined as $p < 0.05$.

Results

TOFA is cytotoxic to cancer cells

TOFA blocks fatty acid synthesis by inhibiting ACCA, the rate-limiting enzyme of the fatty acid synthesis pathway [15,16]. To evaluate the effects of this inhibition on cell growth and survival, we examined the viability of human NCI-H460, HCT-8, and HCT-15 cancer cells exposed to TOFA at 1.0–50.0 μg/ml. As demonstrated in Fig. 1, TOFA showed strong cytotoxicity to all three human cancer cell lines, with an IC_{50} at approximately 5.0, 5.0, and 4.5 μg/ml for NCI-H460, HCT-8, and HCT-15 cells, respectively.

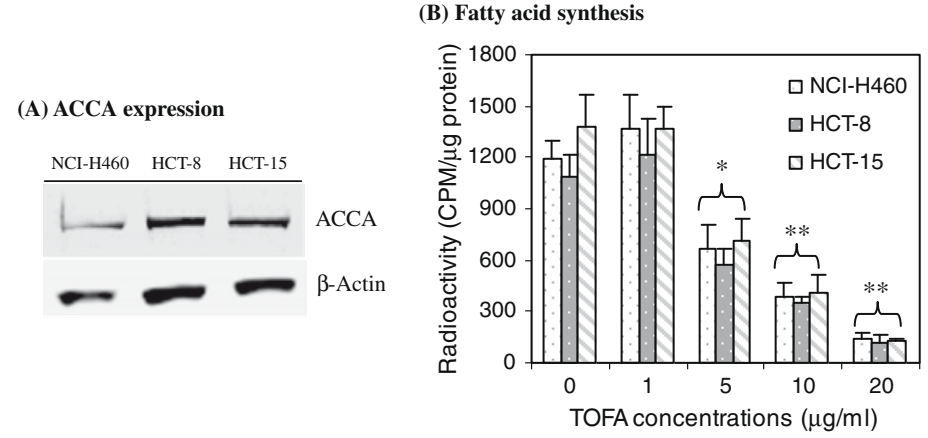

Fig. 2. Inhibition of fatty acid synthesis by TOFA. (A) Western blot. Soluble proteins (100 μg each) were used to detect the ACCA levels in the NCI-H460, HCT-8 and HCT-15 cells, as described in the Materials and methods. Beta-actin was probed to indicate the relative amounts of loaded proteins. (B) Fatty acid synthesis. Cells were exposed to TOFA at the indicated concentrations for 24 h. At 4 h before harvesting, cells were pulsed with 1 μCi 2-^{14}C-labeled acetate per well of 12-well plates and subjected to fatty acid synthesis analysis as described in the Materials and methods. Values represent mean ± SD from three independent measurements. $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to the corresponding control cells without TOFA. ACCA, acetyl-CoA carboxylase-α.